Virulence-associated characteristics of carbapenem-resistant Klebsiella pneumoniae in hospital-acquired infections: results from a hospital in central China

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Abstract

Background Carbapenem-resistant Klebsiella pneumoniae (CRKP) have been a clinically significant pathogen worldwide, but related reports about their virulence features in hospital-acquired infections (HAI) are pretty lacking.

Methods CRKP causing HAI were continuously collected in 2018 from a hospital in central China. Isolates identification and antimicrobial susceptibility test were done using VITEK-2 compact system or MALDI-TOF MS. String test, multilocus sequence typing, carbapenemase genes, virulence genes and capsular antigen genes detection were conducted to understand their phenotype and genetic background. As well as case datas were collected and compared to assess their virulence characteristics.

Results A total of 62 isolates of CRKP from 62 patients with HAI were collected. 41 carbapenemase genistic-confirmed hypervirulent Klebsiella pneumoniae (CR-hvKP) and 21 carbapenem resistant non-hypervirulent Klebsiella pneumoniae (CR-NhvKP) were screened out. Most CRKP causing HAI were ST11 KPC-2 producing strains and mainly causing pneumonia. Only for blaKPC-2 there was a significant difference between CR-hvKP and CR-NhvKP (p<0.001). No significant difference of the two group strains in resistance against amikacin, trimethoprim-sulfamethoxazole, cefepime, ceftazidime, imipenem, piperacillin-tazobactam, colistin and tigecycline were found except levofloxacin (p<0.001), and all strains showed sensitive to tigecycline and colistin. In the CR-hvKP group, IucA (64.5%) were the most commonly detected virulence gene, followed by iroN (48.4%), prmpA2 (30.6%) and prmpA (4.8%), only 1 (2.4%) capsular serotype positive strain and 2 (4.9%) hypermucoviscosity phenotype strains were detected, while no hypermucoviscosity phenotype or capsular antigen gene positive strain was detected in the CR-NhvKP group. And there was no significant difference between the two groups in age, types of infection, departmental distribution, survival time or the final outcome of infection.

Conclusion ST11 KPC-2-producing Klebsiella pneumoniae are most prevalent CRKP in HAI. Virulence gene especially iucA has a high proportion and worth paying attention to. Hypermucoviscous phenotype and virulence-associated capsular serotype in CRKP both have a low prevalence. CRKP harboring virulence genes have a higher expression of KPC-2 and less sensitive to levofloxacin than those harboring no virulence gene, and there is no significant difference for virulence manifestations between the two groups.

Background

There are two types of Klebsiella pneumoniae, classic Klebsiella pneumoniae (cKP) and hypervirulent Klebsiella pneumonia (hvKP) simultaneously prevailing around the world, which pose a great threaten to human health, especially in China. hvKP often cause life-treating community-acquired infections such as severe pneumonia, liver abscesses, meningitis, and endophthalmitis. Reports of infection caused by hvKP have been an increasing trend in recent years. To date, to precisely distinguish hvKP from cKP is unable, but several factors have been found associated with hypervirulence, like hypermucoviscous
phenotype, serotypes, multilocus sequence types (MLSTs), integrative and conjugative elements, and virulence genes. Of all the factors, virulence genes were attached most importance to.[2–5] In a recent study, iucA, plasmid-borne \( rmpA \) gene (\( rmpA \)) and \( rmpA_2 \) demonstrated >0.95 diagnostic accuracy for identifying the hvKP strains.[6] Though hvKP in HAI reported, they were often sensitive to carbapenem.[7–8] Here, we conducted a research on CRKP, comparing the clinical and molecular characteristics between probable CR-hvKP and CR-NhvKP, to have a better understanding of their virulence features in HAI.

Methods

Clinical data collection and bacterial identification

A retrospective case control study of patients with HAI caused by CRKP was conducted. All isolates were continuously collected in 2018 from the clinical microbiology laboratory of Hunan Provincial People's Hospital, a medical institution located in central China. All isolates were identified using VETEK 2-Compact System (bioMérieux, Marcy-l’Étoile, France) or MALDI-TOF MS (bioMérieux, Marcy-l’Étoile, France). The Infection symptoms and hospitalization process of corresponding patients were collected from medical records. Infections were considered as HAI when a new infection developed 48 hours after admission in our study. The demographic datas (gender and age), types of infection, departmental distribution, survival time, and the final outcome of infection of each patient were recorded. Cases without complete outcome information were excluded, as well as duplicate isolates isolated multiple times from the same site in a same patient.

Antimicrobial susceptibility test

The antimicrobial MIC of all isolates was tested by VETEK 2-Compact System, tested antibiotics including amikacin, trimethoprim-sulfamethoxazole, cefepime, ceftazidime, imipenem, piperacillin-tazobactam, levofloxacin, colistin and tigecycline. The results were interpreted according to CLSI.[9] The isolates resistant to tigecycline and imipenem were further confirmed by the E-test method (Biokangtai, Shenzhen, China and Autobio, Zhengzhou, China), and the isolates resistant to colistin were further confirmed by broth dilution method. The MIC of imipenem \( \geq 4 \) mg/L was defined as CRKP.

String test

The hypermucoviscous phenotype was measured by string test. The isolates were incubated with 5% sheep blood medium in a 35°C incubator containing 30% CO\(_2\) for 18 to 24 hours, and then a single colony was gently picked up with a dry 5\( \mu\)l inoculating loop to observe the viscous of the string. If the string length was more than 5 mm, it would be judged as string test positive, as hypermucoviscous phenotype.[10]
MLST

MLST were done according to the protocol and primers on Pasteur Institute MLST website (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html). Seven housekeeping genes of Klebsiella pneumoniae were amplified and sequenced, then submitted to MLST database and analysed.

Carbapenemase genes, virulence genes and capsular antigen genes detection

The collected CRKP were detected by polymerase chain reaction (PCR) method for the carbapenemase genes bla\textsubscript{KPC}, bla\textsubscript{NDM} and bla\textsubscript{IMP} as well as virulence genes iuc\textsubscript{A}, iro\textsubscript{N}, p\textsubscript{rmpA} and p\textsubscript{rmpA\textsubscript{2}} and were done as previously.\textsuperscript{[11][12]} CRKP isolate, which harbored any of the four virulence genes iuc\textsubscript{A}, iro\textsubscript{N}, p\textsubscript{rmpA} or p\textsubscript{rmpA\textsubscript{2}}, could be defined as a CR-hvKP strain, otherwise a CR-NhvKP strain. A total volume was 25 μL of the PCR system, in addition to ddH\textsubscript{2}O, also contained 12.5 μL of 2×PCR super mixture (TransGen Biotech, Beijing, China), and primer F/R 0.2 μL respectively, and DNA template 3 μL. The primer sequence information is shown in table 1. Capsule antigens were detected by amplifying genes encoding for K1, K2, K5, K20, K54 and K57, which were done as previously described.\textsuperscript{[13]} PCR was amplified by BIO-RAD T100 PCR amplifier and the products were electrophoresed on 1% or 0.7% agarose gel. Positive products were sent for sequencing (Qingke, Changsha, China), then were compared with those in the database located at NCBI blast server (http://blast.ncbi.nlm.nih.gov), and analysis was performed using CLC Secquence View (Version 8.0).

Statistical analysis

Datas were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 21.0. Continuous variables were expressed as mean ± SD. The Chi-square test or Fisher’s exact test were used for comparison between groups. The measurement datas were first tested for homogeneity of variance, if the datas were in accordance with the normal distribution, they were compared by independent sample t test, if not, compared by approximate t test. Results with $p<0.05$ were considered significant.

Results

Sample distribution and demographics

A total of 62 isolates of CRKP from 62 patients with HAI were collected, during January 1 to December 31, 2018. Patients stayed in the ICU account for 30.6%, and surgical department 58.1%, internal medicine 8.1%, paediatrics 3.2%, respectively. CRKP were most frequently isolated from sputum samples (40.3%), followed by abdominal drainage fluid (24.2%), biliary drainage fluid (16.2%), blood (12.9%), wound swabs (3.2%), urine (1.6%) and vaginal swabs (1.6%). 41 CR-hvKP and 21 CR-NhvKP were divided based on
virulence genes *iucA*, *iroN*, *rmpA* and *rmpA_2*. CR-hvKP account for 66.1% of all HAI CRKP. 30 males and 11 females were in the CR-hvKP group with an average age of 57.07±18.14 years old, while 14 males and 7 females in the CR-NhvKP group with an average age of 56.86±23.50 years old. Both groups strains had the highest prevalence in people over 50 years old. There was no significant difference in gender construction, age or department distribution between the two groups (*p*>0.05), as details shown in table 2.

**Antimicrobial susceptibility**

All CRKP strains isolated in this research were MDR. In addition to resistance against imipenem, they also exhibited high resistance against cefepime (100%), ceftazidime (100%), piperacillin-tazobactam (98.4%), levofoxacin (83.9%) and trimethoprim-sulfamethoxazole (54.8%), and relative low resistance against amikacin (38.7%). All strains remained great sensitive to tigecycline and colistin and no resistance strain against the two antibiotics was found. There was no significant difference between CR-hvKP and CR-NhvKP strains in the resistance pattern for all of the tested antibiotics except levofloxacin (resistance rate 100.0% and 52.4%, respectively, *p*<0.001). Specific antibiotic resistance datas and comparison results are shown in table 4.

**Carbapenem resistance mechanism**

Of the 62 CRKP strains, 49 isolates harbored *bla*<sub>KPC</sub> gene, all of which were confirmed as *bla*KPC–2, and only one isolate harbored *bla*<sub>IMP</sub> gene, confirmed as *bla*<sub>IMP</sub>–4. No isolate harboring *bla*<sub>NDM</sub> gene was found. 12 isolates harbored non of the above three carbapenemase genes. The positive rate of *bla*KPC–2 gene in CR-hvKP group was significantly higher than that in CR-NhvKP group (95.0% and 47.6%, respectively, *p*<0.001). The detailed information of carbapenem resistance gene detected is shown in table 3.

**MLST genotype**

13 STs were identified among the 62 CRKP strains, including 1 new ST. MLST assigned 79.0% of CRKP isolates to the ST11-clone. ST11 were strongly associated with CR-hvKP (97.6%), followed by one ST2928 strain (2.4%, harbored K54 and *bla*<sub>IMP</sub> gene). ST11 in CR-NhvKP group accounts for 42.9%, and more different STs were found in this group, including 2 ST571, 1 ST15, 1 ST37, 1 ST70, 1 ST101, 1 ST414, 1 ST515, 1 ST1040, 1 ST1779, 1 ST1933 and 1 new ST.

**Virulence-associated features of CR-hvKP and CR-NhvKP**

Only 2 hypermucoviscosity strains were found in the 41 CR-hvKP strains (both of them harbored *bla*KPC–2, *iucA* and *iroN* gene, and one harbored *rmpA_2* gene). Only one capsular antigen gene was detected, which
was K54 (this isolate only harbored \(pmpA_2\) gene, and simultaneously harbored \(bla_{IMP-4}\) gene, string test negative). No K1, K2, K5, K20, K57 positive isolate was found (table 5). The highest positive rate of virulence genes was \(iucA\), 40 isolates harbored \(iucA\) were found and accounted for 64.5% of all CRKP, followed by \(iroN\) (30, 48.4%), \(pmpA_2\) (19, 30.6%), \(pmpA\) (3, 4.8%), respectively. 3 isolates harbored all the four virulence genes (4.8%). No only \(iroN\) positive isolates were isolated. There were no hypermucoviscosity or capsular antigen gene positive strains in the CR-NhvKP group.

**Clinical characteristics comparison between CR-hvKP and CR-NhvKP**

CRKP were responsible for a wide range of infections, including pneumonia (40.3%), abdominal infection (24.2%), biliary tract infection (16.2%), bloodstream infection (12.9%), surgical wound infection (3.2%), urinary tract infection (1.6%) and vaginal infection (1.6%). CR-hvKP and CR-NhvKP strains shared the similar infection types (table 5). Metastatic infections were found from two patient (both were caused by CR-hvKP). There was no significant difference in survival days and mortality of death between the two groups (survival days was 20.14±8.91 days and 22.40±15.03 days, respectively, \(p = 0.750\). Mortality was 17.1% and 23.8%, respectively, \(p = 0.520\)). The results are shown in table 6.

**Discussion**

The prominent feature of hvKP is the ability to cause life-threatening community-acquired infections in healthier individuals and to make the infections severer by metastatic spreading in blood.\[^{14}\] Biomarkers are needed to disinguish hvKP from cKP for diagnosis and treatment as hvKP is undergoing global dissemination. To date, most hvKP strains are hypermucoviscous, belong to serotype K1 or K2, or K5, K20, K54, K57, or specific STs. But these biomarkers can cause mistake when used alone to define a hvKP strain, for not all hvKP having these performances.\[^{15–17}\] Virulence plasmids identified in hvKP, such as \(pK2044\) and \(pLVPK\), with virulence genes harbored, were strongly associated with the hypervirulent phenotype.\[^{2;18–20}\] ST11 hvKP with extremely carbapenem resistance causing fatal outbreak have been reported.\[^{12}\] But the virulence of CR-hvKP seems variable. So far reduced virulence is obvious in colistin resistant CR-hvKP.\[^{21}\] Yen et al. found that ST11 KPC–2-producing CR-hvKP was not maximally virulent in a mouse systemic infection model, even it harbored \(iroBCDN, iucABCD, mpa\) and \(mpA_2\)\[^{20}\]. It suggested that the virulence performance of hvKP not only depending on genetic background, but also related to some other factors, like acquisition of antibiotic resistance and host fighting. What’s more, virulence mechanism and colonization affect still needs to be revealed.

Our results showed that the pneumonia was the most commom infection in both CR-hvKP and CR-NhvKP HAI. Almost all CR-hvKP shared the same ST11 suggesting these strains originated from the same clone. The main mechanism against carbapenem was KPC–2 producation, and ST11 was strongly associated with virulence genes, which were in line with some other researches.\[^{12/22/23}\] Comparison between CR-
hvKP and CR-NhvKP showed a relationship between $\text{bla}_\text{KPC–2}$ and virulence genes. Analysis of antimicrobial susceptibility revealed that the resistance profiles were similar except levofloxacin, CR-hvKP was less sensitive to this antibiotic. All CRKP in this study remained sensitive to tigecycline and colistin, continuous monitoring will be conducted for the alert that the combination of multidrug resistance and enhanced virulence has the potential to cause the next clinical crisis.

Of the 41 CR-hvKP isolates, 2.4% showed capsular serotype K54 and 4.9% showed hypermucoviscosity, both were lower than other previously reported results,\textsuperscript{[7:8]} and no K1 or K2 strain was found in both groups. We analysed the clinical characteristics of CR-hvKP by comparing with CR-NhvKP through age, types of infection, departmental distribution, survival time, and infection outcome, and there were no significant difference between the two groups, suggesting impaired virulence of CR-hvKP in HAI even though harboring virulence genes. A recent study conducted in India found a similar result.\textsuperscript{[8]} For a lack of method that can precisely differentiate hvKP from cKP, the CR-hvKP might be overestimated in this study and there was a lack of model validation. But it is worth noting that there was a high prevalence of virulence genes in CRKP, especially $\text{iucA}$. Patients who have undergone HAI by CR-hvKP possibly have CR-hvKP colonized, whom might become potential sources of infection for severer community-acquired infections.

Currently, the treatment for CR-hvKP HAI is kontty and the antibiotics available are extremely limited. Discovering and subsequently quarantining are critical measures. Contact precautions are necessary especially in ICU. Screening for CR-hvKP of respiratory specimens can be conducted in older patient. Also, more information about hvKP needs to be discovered to help clinical microbiology laboratory carrying out measures to identify this variant pathogen.

**Conclusions**

ST11 KPC–2-producing *Klebsiella pneumoniae* is most prevalent CRKP in HAI. Virulence gene especially $\text{iucA}$ has a high proportion and worth paying attention to. Hypermucoviscous phenotype and virulence-associated capsular serotype in CRKP both has a low prevalence. CRKP harboring virulence genes have a higher expression of KPC–2 and less sensitive to levofloxacin than those harboring no virulence gene, and there is no significant difference for virulence manifestations between the two groups.

**Declarations**

**Ethics approval and consent to participate**

This study was approved by Ethics Committee of Hunan Provincial People's Hospital, The First Affiliated Hospital of Hunan Normal University and gained patients’ consent.

**Consent for publication**
Not applicable.

**Availability of data and materials**

All data generated or analysed during this study are included in this published article.

**Competing interests**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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**Authors' contributions**

Liangyi XIE and Pengwen OUYANG conceived and designed the work. Pengwen OUYANG, Liangyi XIE, Bin JIANG, Juan WANG, Na PENG, Jianrong YE, Yiping CHEN performed the survey. Pengwen OUYANG analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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**References**


Tables

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